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03/06/98 JC582 U.S. PTO

File No.: **1225/0C675-US2**

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REISSUE OF US PATENT NO. 5,290,551 TO BERD

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

Enclosed is an application for a reissue of United States Patent No. 5,290,551 to Berd, entitled "TREATMENT OF MELANOMA WITH A VACCINE COMPRISING IRRADIATED AUTOLOGOUS MELANOMA TUMOR CELLS CONJUGATED TO A HAPTEN." The patent issued March 1, 1994, and is assigned to Thomas Jefferson University, Philadelphia, P.A. The reissue application includes:

1. Specification and 2 original claims
2. Preliminary Amendment
3. Declaration by Inventor under 37 C.F.R. §1.175
4. Assent to Reissue by Assignee Thomas Jefferson University
5. Offer to Surrender the Original Letters Patent by Assignee (37 C.F.R. §1.178) and a Submission Establishing Ownership under 37 C.F.R. §3.73(b)
6. Powers of Attorney by Inventor
7. Powers of Attorney by Assignee

Check in the amount of $ 790.00 ($790.00 filing fee). This fee covers 2 independent claim (1 original and 1 new) and 18 dependent claims (1 original and 17 new). The Commissioner is authorized to charge any deficiency in this amount or to credit any overpayment to Deposit Account No. 04-0100.

Respectfully submitted,

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D B Peck

Name (Print)

Signature

1225/0C675-US2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: David BERD

Serial No.: Not Yet Assigned (For Reissue of U.S. Patent No. 5,290,551)

Filed: Concurrently Herewith

For: **TREATMENT OF MELANOMA WITH A VACCINE COMPRISING IRRADIATED AUTOLOGOUS MELANOMA TUMOR CELLS CONJUGATED TO A HAPTEN**

Assignee: Thomas Jefferson University

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

REISSUE SPECIFICATION AND CLAIMS

Sir:

Attached is a column-by-column copy of U.S. Patent No. 5,290,551 for "TREATMENT OF MELANOMA WITH A VACCINE COMPRISING IRRADIATED AUTOLOGOUS MELANOMA TUMOR CELLS CONJUGATED TO A HAPTEN" granted to David Berd on March 1, 1994.

US005290551A

United States Patent [19]

Berd

[11] Patent Number: 5,290,551

[45] Date of Patent: Mar. 1, 1994

[54] TREATMENT OF MELANOMA WITH A VACCINE COMPRISING IRRADIATED AUTOLOGOUS MELANOMA TUMOR CELLS CONJUGATED TO A HAPTEN

[75] Inventor: David Berd, Wyncote, Pa.

[73] Assignee: Thomas Jefferson University, Philadelphia, Pa.

[21] Appl. No.: 985,334

[22] Filed: Dec. 4, 1992

Related U.S. Application Data

[63] Continuation of Ser. No. 520,649, May 8, 1990, abandoned.

[51] Int. Cl.[5] A61K 39/00; A61K 37/66

[52] U.S. Cl. 424/88; 424/85.2

[58] Field of Search 424/88, 85.2

[56] References Cited

U.S. PATENT DOCUMENTS

4,108,983 8/1978 Wallack 424/89

OTHER PUBLICATIONS

Bystryn, J-C., Journal of Immunology, vol. 120 (1), pp. 96–101, (1978).
Mitchison, *Transplant. Proc.* 2:92–103 (1970).
Fujiwara et al., *J. Immunol.* 132:1571–1577 (1984a).
Flood et al., *J. Immunol.* 138:3573–3579 (1987).
Fujiwara et al., *J. Immunol.* 133:510–514 (1984b).
Berd et al., *Cancer Res.* 42:4862–4866 (1982).
Berd et al., *Cancer Res.* 44:1275–1280 (1984a).
Berd et al., *Cancer Res.* 46:2572–2577 (1986).
Berd et al., *Cancer Invest.* 6:335–347 (1988a).
Berd et al., *Cancer Res.* 44:5439–5443 (1984b).
Berd et al., *Cancer Res.* 47:3317–3321 (1987).
Berd et al., *Cancer Res.* 48:1671–1675 (1988b).
Berd et al., *Proc. Amer. Assoc. Cancer Res.* 29:408 (#1626) (1988c).
Topalian et al., *J. Clin. Oncol.* 6:839–853 (1988).
Lotze et al., *J. Biol. Response* 3:475–482 (1982).
West et al., *New Engl. J. Med.* 316:898–903 (1987).
Talmadge et al., *Cancer Res.* 47:5725–5732 (1987).
Meuer et al., *Lancet* 1:15–18 (1989).
Miller and Claman, *J. Immunol.* 117:1519–1526 (1976).

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[57] ABSTRACT

The invention is a haptenized tumor vaccine for the treatment of cancer. Treatment of cancer patients with an autologous, vaccine preceded by low dose cyclophosphamide (CY) induces delayed-type hypersensitivity (DTH) to melanoma cells, and in some cases, regression of metastatic tumors. The efficiency of the process has been increased by immunizing with tumor cells conjugated to the hapten such as DNP, TNP or AED.

Additional embodiments of the vaccine include: 1) combining the vaccine with immunomodulating drugs, such as, interleukin-2 (IL2); and 2) purifying the active components of the vaccine by extracting antigens from cancer cells to produce a chemically-defined, haptenated vaccine. The treatment may also be extended to include other types of human cancer.

2 Claims, No Drawings

TREATMENT OF MELANOMA WITH A VACCINE COMPRISING IRRADIATED AUTOLOGOUS MELANOMA TUMOR CELLS CONJUGATED TO A HAPTEN

This is a continuation of application Ser. No. 520,649, filed May 8, 1990.

INTRODUCTION

The invention described herein was made in the course of work under a grant or award from an NIH Cancer Research grant.

This invention was disclosed in a Disclosure Document filed Apr. 18, 1990, which is now abandoned.

BACKGROUND OF THE INVENTION

It was theorized in the 1960's that tumor cells bear specific antigens (TSA) which are not present on normal cells and that the immune response to these antigens might enable an individual to reject a tumor. It was later suggested that the immune response to TSA could be increased by introducing new immunological determinants on cells. Mitchison, *Transplant. Proc.* 2:92–103 (1970). Such a "helper determinant", which can be a hapten, a protein, a viral coat antigen, a transplantation antigen, or a xenogenous cell antigen, could be introduced into a population of tumor cells. The cells would then be injected into an individual who would be expected to be tolerant to the growth of unmodified tumor cells. Clinically, the hope was that an immunologic reaction would occur against the helper determinants, as a consequence of which the reaction to the accompanying TSA is increased, and tumor cells which would otherwise be tolerated are destroyed. Mitchison (1970) also suggests several modes of action of the helper determinants including 1) that the unmodified cells are merely attenuated, in the sense that their growth rate is slowed down or their susceptability to immunologic attack increased; 2) that helper determinants merely provide points of attack and so enable the modified cells to be killed by an immune response not directed against TSA; 3) that the helper determinants have an adjuvant action such as binding to an antibody or promoting localization of the cells in the right part of the body for immunization, in particular, in lymph nodes.

Fujiwara et al., *J. Immunol.* 132:1571–1577 (1984a) showed in a murine system that tumor cells conjugated with the hapten, trinitrophenyl (TNP), could induce systemic immunity against unmodified tumor cells, provided that the animals were first sensitized to the hapten in the absence of hapten-specific suppressor T cells. Spleen cells from the treated mice completely and specifically prevented the growth of tumors in untreated recipient animals. Flood et al., *J. Immunol.* 138:3573–3579 (1987) showed that mice immunized with a TNP-conjugated, ultraviolet light-induced "regressor" tumor were able to reject a TNP-conjugated "progressor" tumor that was otherwise non-immunologic. Moreover, these mice were subsequently resistant to challenge with unconjugated "progressor" tumor. In another experimental system, Fujiwara et al., *J. Immunol. 133:510–514* (1984b) demonstrated that mice sensitized to trinitrochlorobenzene (TNCB) after cyclophosphamide (CY pretreatment could be cured of large (10 mm) tumors by in situ haptenization of tumor cells; subsequently, these animals were specifically resistant to challenge with unconjugated tumor cells.

The common denominator of these experiments is sensitization with hapten in a milieu in which suppressor cells are not induced. Spleen cells from CY-pretreated, TNCB-sensitized mice exhibited radioresistant "amplified helper function" i.e., they specifically augmented the in vitro generation of anti-TNP cytotoxicity. Moreover, once these amplified helpers had been activated by in vitro exposure to TNP-conjugated autologous lymphocytes, they were able to augment cytotoxicity to unrelated antigens as well, including tumor antigens (Fujiwara et al., 1984b). Flood et al. (1987) showed that this amplified helper activity was mediated by T cells with the phenotype Lyt-1+, Lyt-2−, L3T4+, I−J+ and suggests that these cells were contrasuppressor cells, a new class of immunoregulatory T cell.

Immunotherapy of patients with melanoma has shown that administration of CY, either high dose (1000 mg/M^2) or low dose (300 mg/M^2), three days before sensitization with the primary antigen keyhole limpet hemocyanin (KLH) markedly augments the acquisition of delayed type hypersensitivity (DTH) to that antigen (Berd et al., *Cancer Res.* 42:4862–4866 (1982); *Cancer Res.* 44:1275–1280 (1984a)). Low dose CY pretreatment allows patients with metastatic melanoma to develop DTH to autologous melanoma cells in response to injection with autologous melanoma vaccine (Berd et al., *Cancer Res.* 46:2572–2577 (1986)). The combination of low dose CY and vaccine can produce clinically important regression of metastatic tumor (Berd et al. (1986); *Cancer Invest.* 6:335–347 (1988a)). CY administration results in reduction of peripheral blood lymphocyte non-specific T suppressor function (Berd et al., *Cancer Res.* 44:5439–5443 (1984b); *Cancer Res.* 47:3317–3321 (1987)), possibly by depleting CD4+, CD45R+ suppressor inducer T cells (Berd et al., *Cancer Res.* 48:1671–1675 (1988b)). The anti-tumor effects of this immunotherapy regimen appear to be limited by the excessively long interval between the initiation of vaccine administration and the development of DTH to the tumor cells (Berd et al., *Proc. Amer. Assoc. Cancer Res.* 29:408 (#1626) (1988c)). Therefore, there remains a need to increase the therapeutic efficiency of such a vaccine to make it more immunogenic.

Most tumor immunologists now agree that getting T lymphocytes, the white cells responsible for tumor immunity, into the tumor mass is a prerequisite for tumor destruction by the immune system. Consequently, a good deal of attention has been focused on what has become known as "TIL" therapy, as pioneered by Dr. Stephen Rosenberg at NCI. Dr. Rosenberg and others have extracted from human cancer metastases the few T lymphocytes that are naturally present and greatly expanded their numbers by culturing then in vitro with Interleukin-2 (IL2), a growth factor for T lymphocytes Topalian et al., *J.Clin. Oncol.* 6:839–853 (1988). However this therapy has not been very effective because the injected T cells are limited in their ability to "home" to the tumor cite.

The ability of high concentrations of IL2 to induce lymphocytes to become non-specifically cytotoxic killer cells has been exploited therapeutically in a number of studies (Lotze et al., *J. Biol. Response* 3:475–482 (1982); West et al., *New Engl. J. Med.* 316:898–903 (1987)). However, this approach has been limited by the severe toxicity of high dose intravenous IL2. Less attention has been given to the observation that much lower concentrations of IL2 can act as an immunological adjuvant by inducing the expansion of antigen activated T

cells (Talmadge et al., *Cancer Res.* 47:5725–5732 (1987); Meuer et al., *Lancet* 1:15–18 (1989)). Therefore, there remains a need to understand and attempt to exploit the use of IL2 as an immunological adjuvant.

SUMMARY OF THE INVENTION

The present invention is a haptenized tumor vaccine for the treatment of cancer. Treatment of cancer patients with a haptenized tumor vaccine, preceded by low dose cyclophosphamide (CY) has been found to induce delayed type hypersensitivity (DTH) to melanoma cells, and in some cases, regression of metastatic tumors. The efficiency of the process has been increased by immunizing with tumor cells conjugated to a hapten such as, DNP, TNP, or N-Iodoacetyl-N'-(5-sulfonic 1-naphtyl) ethylene diamine (AED). Additional embodiments of the vaccine include: 1) combining the vaccine with immunomodulating drugs such as Interleukin-2; and 2) purifying the active components of the vaccine by extracting antigens from the cancer cells to produce a chemically-defined, haptenized vaccine.

DETAILED DESCRIPTION OF THE INVENTION

The invention is a form of cancer immunotherapy that involves injecting patients with a novel tumor vaccine. Patients with metastatic melanoma are immunized to the chemical dinitrophenyl (DNP) by application of dinitrofluorobenzene (DNFB) to the skin. Two weeks later, they are injected with a vaccine consisting of the patient's own cancer cells that have been irradiated and haptenized (chemically linked) to DNP. The vaccine is reinjected every 4 weeks. The drug, cyclophosphamide (CY) is administered 3 days prior to each vaccine administration to augment the immune response to the tumor cells.

The vaccine consists of $10–25 \times 10^6$ live, DNP-conjugated tumor cells suspended in 0.2 ml Hanks solution to which is added *Bacille Calmette-Guerin* (BCG) 0.1 ml. The mixture is injected intradermally into 3 contiguous sites on the upper arms or legs, excluding limbs ipsilateral to a lymph node dissection.

The vaccine is prepared as follows. Tumor masses are processed as described by Berd et al. (1986). The cells are extracted by enzymatic dissociation with collagenase and DNAse by mechanical dissociation, frozen in a controlled rate freezer, and stored in liquid nitrogen until needed. On the day that a patient is to be skin tested or treated, the cells are thawed, washed, and irradiated to 2500 R. They are washed again and then suspended in Hanks balanced salt solution without phenol red. Conjugation of the prepared melanoma cells with DNP is performed by the method of Miller and Claman, *J. Immunol.* 117:1519–1526 (1976), which involves a 30 minute incubation of tumor cells with DNFB under sterile conditions, followed by washing with sterile saline.

Other useful haptens include TNP and AED which may be chemically linked to the tumor cells.

Human cancer vaccines have been developed and tested by a number of workers. Although they can sometimes induce weak immunity to a patient's cancer, they rarely cause tumor regression. With the DNP-vaccine of the present invention, the development of inflammatory responses in metastatic tumors was surprisingly found. The tumor becomes reddened, warm and tender. Microscopically, infiltration of T lymphocytes into the tumor mass is observed. Therefore, this ap-

proach, which increases the inflammatory response and the number and capacity of lymphocytes entering the tumor, is a significant advance in the art.

It has also been found that administration of an immunomodulating drug, such as IL2, further enhances the efficacy of the present invention. In this embodiment, IL2 is given following the vaccine injection. Administration of IL2 to patients with inflammatory responses causes the T lymphocytes within the tumor mass to proliferate and become more active. The increased T cell numbers and functional capacity leads to immunological destruction of the tumors.

Recently a new preparation of IL2 has become available, which is covalently linked to polyethylene glycol (PEG). PEG-IL2 has a much longer pharmacological half-life than unmodified IL2 i.e., weekly administration results in sustained blood levels (Investigator's Brochure, Cetus Corporation). Furthermore, the toxicity of weekly administration of PEG-IL2 is mild when the weekly dose is below 1×10^6 IU/M^2. It was found that the administration of low dose IL2 to patients whose tumor have become infiltrated with activated T cells results in expansion of those cells and more potent anti-tumor effects. Patients with metastatic melanoma were treated using an immunotherapy regimen with the following components: 1) vaccine consisting of autologous tumor cells conjugated to DNP; 2) low dose CY pretreatment; and 3) PEG-IL2 given weekly following vaccine injection. Patients were evaluated to determine whether tumor regression had occurred, to monitor tumor inflammatory responses, and to measure DTH to autologous melanoma cells, DNFB (the form of DNP used for skin sensitization), DNP-conjugated autologous lymphocytes, diluent (Hanks solution), PPD, and recall antigens (candida, trichophyton, and mumps). Patients who are considered to be deriving benefit (clinical or immunological) from the therapy are continued in the immunotherapy regimen. Subsequent vaccines may be given without CY.

In another embodiment, a vaccine comprising chemical extracts of cancer cells conjugated to a hapten and mixed with an immunological adjuvant, such as BCG, is used. Chemical extracts of the cancer cells are prepared by protein extraction techniques from the cancer cells, followed by antigen assays to determine the most effective antigen(s) for patient treatment. The methodology for developing pharmaceuticals based on such purified active components of such a vaccine is well known in the art.

The invention is further illustrated by means of the following examples which are meant to be illustrations only and are not intended to limit the present invention to these specific embodiments.

EXAMPLE 1

Sixty-four patients were treated with metastatic melanoma using a melanoma vaccine preceded by low dose cyclophosphamide (CY) and monitored for immunological effects and anti-tumor activity. On day 0, the patients were given CY 300 mg/M^{2}IV. Three days later, they were injected intradermally with vaccine consisting of $10–25\times10^6$ autologous, cryopreserved, irradiated (2500 R) tumor cells mixed with BCG; the tumor cells were obtained by dissociation of metastatic masses enzymatically (collagenase and DNAse). This treatment sequence was repeated every 28 days.

The toxicity of the therapy was limited to a local inflammatory response at the injection site and mild

nausea and vomiting following CY. There were 40 evaluable patients with measurable metastases; 5 had responses—4 complete and 1 partial. The median duration of response was 10 months (7–84 +months). Regression occurred not only in skin and nodal metastases, but also in lung and liver metastases. In 6 additional patients, we observed an anti-tumor response that seemed peculiar to this vaccine therapy, i.e., the regression of metastatic lesions that appeared after the immunotherapy was begun. In 3 patients this "delayed" regression occurred in two or more tumors.

Delayed-type hypersensitivity (DTH) to autologous, mechanically-dissociated melanoma cells was detectable in only 16% of patients before treatment, as compared with in 46%, 56% and 73% of patients on days 49, 161 and 217, respectively. The increases in DTH following immunotherapy were statistically significant by a non-independent t-test; day 0 vs. day 49, $p.<0.001$; day 0 vs. day 161, $p<0.001$; day 0 vs. day 217, $p=0.02$. Overall, 26/43 patients (61%) exhibited a positive DTH response (5 mm or>induration) to autologous melanoma cells at some time point. Patients also developed strong DTH to the enzymes used to prepare the tumor cell suspensions: of 24 patients tested for DTH with a mixture of collagenase and DNase (each at 1 ug/ml) after two vaccine treatments, 21 (88%) had responses>5 mm induration. Anti-tumor responses to the vaccine were strongly associated with DTH to mechanically-dissociated, autologous melanoma cells, as indicated by three observations: 1) 8/10 patients who exhibited tumor regression had positive DTH; 2) in post-surgical adjuvant patients, there was a highly significant correlation between the intensity of DTH to autologous melanoma cells and the time to recurrence of tumor ($r=0.680$, $p<0.001$); 3) nine patients who developed DTH to the autologous melanoma cells in their original vaccine ("old" tumor) developed new metastases ("new" tumor) that did not elicit DTH or elicited a much smaller response.

In three cases we were able to excise regressing tumors for histological examination; such tumors were characterized by an intense infiltration of lymphocytes. In contrast, tumors excised from these patients before immunotherapy consisted of homogeneous masse of malignant cells without significant lymphocytic infiltration.

This study shows that the use of CY allows the development of an immune response to melanoma-associated antigens in cancer-bearing patients.

EXAMPLE 2

Patients with metastatic melanoma were sensitized to DNP by topical application of dinitrochlorobenzene (DNCB) or dinitrofluorobenzene (DNFB). Two weeks later they were injected with a vaccine consisting of $10–25\times10^6$ autologous, irradiated melanoma cells conjugated to DNP and mixed with BCG. CY 300 mg/M^2IV was given 3 days before DNCB (or DNFB) or vaccine. Of 4 patients evaluable so far, 3 have developed a striking inflammatory response in tumor masses after 2 vaccine treatments (8 weeks). Patient #1 developed erythema and swelling in the >50 large (1–3 cm) dermal metastases on her leg and lower abdomen, followed by ulceration and drainage of necrotic material, and some are beginning to regress. Biopsy showed infiltration with CD4+CD8+T lymphocytes. Patient #2 developed erythema and swelling in the skin of her lower abdomen and groin overlying large (8 cm) nodal

masses. These have not yet regressed, but have changed in consistency from rockhard to fluctuant. Patient #3 exhibited moderate erythema in the skin overlying 3 subcutaneous metastases. All 3 patients have developed DTH to both DNCB and to DNP conjugated autologous lymphocytes.

EXAMPLE 3

Fifteen patients (including 3 patients from Example 2) were treated with metastatic melanoma using a novel form of immunotherapy, i.e., tumor cell vaccine conjugated to DNP. Patients were sensitized to DNP by topical application o 5% dinitrochlorobenzene. Then every 4 weeks they received cyclophosphamide 300 mg/M^2 followed 3 days later by injection of $10–25 \times 10^6$ autologous, irradiated melanoma cells conjugated to DNP. Most patients (92%) developed delayed-type hypersensitivity (DTH) to DNP-conjugated autologous lymphocytes or tumor cells (mean DTH = 17 mm). The vaccine induced a striking inflammatory response in sc and nodal metastases in 11/15 patients, consisting of erythema, swelling, warmth, and tenderness around tumor masses, and, in one case, purulent drainage. Biopsies showed infiltration with lymphocytes, which, by immunopathological and flow cytometric analyses, were mainly CD3+, CD4−, CD8+, HLA−DR+T cells. The melanoma cells in these tissues strongly expressed ICAM-1, which serves as an adhesion molecule for T cells. Thus, DNP-vaccine seems to induce a degree of anti-melanoma immunity not seen with previously tested immunotherapy.

EXAMPLE 4

Patients with metastatic melanoma are sensitized to the hapten, 1-fluoro-2,4-dinitrobenzene (DNFB). This is the form of DNP used for skin sensitization. They are then treated with the following active immunotherapy regimen: low dose CY (obtained from Bristol Laboratories (Evansville, Ind.) which is reconstituted in sterile water and the proper dosage administered by rapid IV infusion) followed 3 days later by intradermal injection of a vaccine consisting of autologous, irradiated melanoma cells conjugated to DNP and mixed with BCG (Glaxo strain (Danish 1077) obtained from Glaxo (Greenford, England) and distributed by Quad Pharmaceuticals Inc. (Indianapolis, Ind.). The freeze-dried material is reconstituted with 1 ml sterile water; then 0.1 ml (0.8–2.6 million organisms) is drawn up, mixed with the vaccine and injected. The cyclophosphamide-vaccine sequence is repeated on days 28–31. Patients are evaluated on day 51 for tumor regression, tumor inflammatory response, and delayed-type hypersensitivity to autologous melanoma cells. They then receive three weekly injections of PEG-IL2 given as an IV bolus. PEG-IL2 was obtained from Cetus Corporation (Emeryville, Calif.). It is prepared by covalently binding PEG (6–7 Kd MW) to human recombinant IL2. The specific activity is approximately 6×10^6 IU/mg. PEG-IL2 is supplied as a sterile lyophilized product. The material is reconstituted with 1.2 ml sterile water, diluted on 50 cc 0.9% Sodium Chloride Injection, USP, and infused intravenously over 2–5 minutes. Another evaluation is performed on day 79. The entire cycle, without CY is repeated on day 84.

What is claimed:

1. A vaccine useful for the treatment of melanoma comprising irradiated autologous melanoma cells conjugated to a hapten, said hapten selected from the group

consisting of dinitrophenyl, trinitrophenyl, and N-iodoacetyl-N'-5 sulfonic 1-naphtyl ethylene diamine; and mixed with an immunological adjuvant, wherein said immunological adjuvant is *Bacille Calmette-Guerin.*

2. A method for treating melanoma comprising ad-

ministering cyclophosphamide followed by intradermal administration of a therapeutically effective amount of the vaccine of claim 1.

* * * * *

1515

Tumor inflammatory response induced by immunization with autologous melanoma cells conjugated to dinitrophenol(DNP). D. Berd, M.J. Mastrangelo, C. Green, C. Clark, and E. Hart. Thomas Jefferson University, Philadelphia, PA 19107.

Treatment of melanoma patients with an autologous vaccine preceded by low dose cyclophosphamide (CY) induces delayed-type hypersensitivity (DTH) to melanoma cells, and in some cases, regression of metastatic tumors. Now, we are attempting to increase the efficiency of the process by immunizing with tumor cells conjugated to the hapten, DNP. Patients with metastatic melanoma were sensitized to DNP by topical application of dinitrochlorobenzene (DNCB). Two weeks later, they were injected with a vaccine consisting of 10-25x10(6) autologous, irradiated melanoma cells conjugated to DNP and mixed with BCG. CY 300 mg/M^2 IV was given 3 days before DNCB or vaccine. Of 4 patients evaluable so far, 3 have developed a striking inflammatory response in tumor masses after 2 vaccine treatments (8 weeks). Patient #1 developed erythema and swelling in the >50 large (1-3 cm) dermal metastases on her leg and lower abdomen, followed by ulceration and drainage of necrotic material, and some are beginning to regress. Biopsy showed infiltration with CD4+ and CD8+ T lymphocytes. Patient #2 developed erythema and swelling in the skin of her lower abdomen and groin overlying large (8 cm) nodal masses. These have not yet regressed, but have changed in consistency from rock-hard to fluctuant. Patient #3 exhibited moderate erythema in the skin overlying subcutaneous metastases. All 3 patients have developed DTH to both DNCB and to DNP-conjugated autologous lymphocytes. Although these results are preliminary, they suggest that this new strategy may represent a significant advance in the immunotherapy of human melanoma.

1516

Inhibition of Tumor-Induced Suppressor T Lymphocyte (Ts) Activity by Murine Interferon Beta (IFN-B). Deepak M. Sahasrabudhe, University of Rochester Cancer Center, Rochester, NY, 14642

In some tumor models inhibition of Ts-activity is a prerequisite to successful immunotherapy. Based on our data in the DNFB model (J Exp Med 166:1573, 1987) the effect of IFN-B on P815 mastocytoma-induced Ts-activity was evaluated.

In this model, concomitant antitumor immunity (Tc) peaks by Day 10 and is down regulated by Ts by Day 15. Cytotoxicity generated after a mixed lymphocyte tumor culture (MLTC) correlates with in vivo immunity and suppression of cytotoxicity correlates with in vivo Ts-activity.

Tumors were initiated by injecting 2 x 10^6 P815 cells subcutaneously on Day 1. IFN-B (10U, 1000U, 5000U) or buffer were injected i.v. every other day x 6 doses starting on Day 5. On Day 16, MLTC's were set up. Five days later a cytotoxicity assay was performed against ^{51}Cr labelled P815 cells. % specific lysis is shown. Numbers in parenthesis represent the dose of IFN-B.

		Tc +		Tc		Tc		Tc		Tc
E:T	Tc	Naive	Ts	+Ts	Ts (10)	+Ts (10)	Ts (1000)	+Ts (1000)	Ts (5000)	+Ts (5000)
50:1	88	81	0	19	6	22	23	20	81	84
25:1	84	76	0	12	2	21	1	21	63	75
12:1	78	79	2	15	3	24	6	23	58	81
6:1	70	69	1	7	0	9	0	20	38	64
3:1	56	55	0	8	1	13	0	12	21	48

Treatment with IFN-B 5000U every other day x 6 doses abrogated Ts-activity without adversely affecting cytotoxicity. IFN-B may be a useful adjunct in the immunotherapy of selected tumors.

1517

Anti-idiotype monoclonal antibody immunization therapy of cutaneous T cell lymphoma. Chatterjee, M., Foon, K., Seon, B.K., Barcos, M. and Kohler, H., Roswell Park Mem. Inst. Buffalo, NY 14263, and UCSD, San Diego, CA 92161.

Cutaneous T cell lymphoma (CTCL) is an indolent non-Hodgkin's lymphoma which is not cured by standard therapies once it reaches advanced stage. A novel approach to therapy is to use internal image anti-idiotype (Id) mAb as antigen (Ag) substitute for the induction of immunity. We have generated anti-Id mAb (Ab2) binding to a hybridoma SN2 (Ab1), which recognizes a unique glycoprotein, gp37, expressed by a subset of human leukemic T cells (J. Immunol. 139:1354, 1987). At least 2 of these Ab2 may indeed carry the internal image of the gp37 Ag (J. Immunol. 141:1398, 1988). Recently, we investigated the distribution of gp37 Ag by a sensitive immunoperoxidase staining method using mAb SN2. SN2 had a high specificity for T-leukemia/lymphoma cells and did not react with any normal adult tissues tested including thymus, lymphocytes, bone marrow cells, spleen, liver, kidney, lung, brain, heart, etc. CTCL cells from 5/6 out of 6 patients were strongly positive for gp37 Ag with intense surface membrane staining. The binding of radiolabeled SN2 to CTCL cells was studied for inhibition in the presence of the anti-Id mAbs 4EA2 and 4DC6 which mimic the gp37 Ag. Both clones inhibited the binding 100% and 80% respectively at a concentration of 50 ng. We also generated a murine Ab3 mAb (anti-anti-Id) by immunizing mice with the anti-Id mAb (Ab2). This Ab3 mAb reacts with CTCL cells in an identical fashion as the original Ab1 (SN2). Collectively, these data suggest that Ab2 4EA2 and 4DC6 may be useful for active immunotherapy of CTCL patients. We plan to study the CTCL patients in a phase I clinical trial to determine the effects of this type of therapy on various components of the immune system (both humoral and cellular) and try to identify the criteria to select patients who may benefit from anti-idiotype vaccine therapy.

1518

Syngeneic murine monoclonal antiidiotypes bearing the internal image of a human breast cancer associated antigen. J. Schmitz and H. Ozer. The Dept. of Microbiology, S.U.N.Y. at Buffalo, Buffalo, NY 14214 and the Division of Medical Oncology, The Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

According to Jerne's network theory, some antiidiotypes (Ab2) mimic external antigens recognized by specific antibodies (Ab1) and may be used in place of antigen for immunization. The murine monoclonal antibody F36/22 (IgG3, κ), specific for ductal carcinoma antigen (DCA) was used to generate syngeneic monoclonal antiidiotypes bearing the internal image of DCA. Female BALB/c mice were inoculated intraperitoneally every other week with 100 μg of F36/22 coupled to keyhole limpet hemocyanin; the first time in complete Freund's adjuvant and subsequently in incomplete adjuvant. Splenic lymphocytes were fused with the murine cell line P3X63 Ag8.653 3 days after the fourth immunization using 50% polyethylene glycol (m.w. 3,350). Two hybrids, MTO-1 and MTO-2, were selected based on the ability of culture supernatants to bind to F36/22 but not to the control antibody 2A31F6 (IgG3, κ) in an enzyme linked immunosorbent assay (ELISA) and cloned by limiting dilution. Paratope specificity of Ab2 was demonstrated in two ELISA assays. First, the binding of labeled F36/22 to DCA was inhibited 100% and 75% by 1.6 μg of MTO-2 and MTO-1 respectively. Second, the binding of labeled Ab2 to Ab1 was inhibited by purified DCA. MTO-1 neither enhances nor inhibits the binding of labeled MTO-2 to Ab1 although in the presence of MTO-2, binding of labeled MTO-1 is enhanced by 100% indicating that these Ab2 recognize distinct idiotopes. Rabbits immunized biweekly with MTO-1 or MTO-2 developed antibodies that bound specifically to DCA demonstrating that MTO-1 and MTO-2 bear the internal image of DCA. These data suggest that MTO-1 and MTO-2 could potentially be utilized to immunize high risk patients against progression or development of DCA positive tumors.

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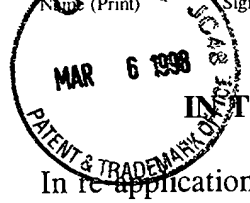

File No.: **1225/0C675-US2**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: David BERD

Serial No: To Be Assigned (For Reissue of U.S. Patent No. 5,290,551)

Filed: Concurrently Herewith

For: **TREATMENT OF MELANOMA WITH A VACCINE COMPRISING IRRADIATED AUTOLOGOUS MELANOMA TUMOR CELLS CONJUGATED TO A HAPTEN**

Assignee: Thomas Jefferson University

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

POWER OF ATTORNEY

Thomas Jefferson University hereby appoints the following attorneys and/or agents to prosecute this reissue patent application and to transact all business in the Patent and Trademark Office connected herewith:

William F. Dudine, Reg. No. 20,569; Michael J. Sweedler, Reg. No. 19,937; S. Peter Ludwig, Reg. No. 25,351; Paul Fields, Reg. No. 20,298; Joseph B. Lerch, Reg. No. 26,936; Melvin C. Garner, Reg. No. 26,272; Ethan Horwitz, Reg. No. 27,646; Beverly B. Goodwin, Reg. No. 28,417; Adda C. Gogoris, Reg. No. 29,714; Martin E. Goldstein, Reg. No. 20,869; Bert J. Lewen, Reg. No. 19,407; Henry Sternberg, Reg. No. 22,408; Peter C. Schechter, Reg. No. 31,662; Robert Schaffer, Reg. No. 31,194; David R. Francescani, Reg. No. 25,159, and Nada Jain, Reg. No. 41,431 all of Darby & Darby, P.C., 805 Third Avenue, New York, NY, 10022.

Thomas Jefferson University

Date: March 5, 1998

By: _____
JUSSI J. SAUKKONEN, MD
Name, Title
Dean, College of Graduate Studies and
Vice President for Science Policy, Technology
Development and International Affairs

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D B Peck

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Date: 3/5/98

David Berd

David BERD

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OFFER TO SURRENDER UNDER 37 C.F.R. §1.178 AND A SUBMISSION ESTABLISHING OWNERSHIP UNDER 37 C.F.R §3.73(b)

Thomas Jefferson University, who is now the sole owner of U.S. Patent No. 5,290,551 for "TREATMENT OF MELANOMA WITH A VACCINE COMPRISING IRRADIATED AUTOLOGOUS MELANOMA TUMOR CELLS CONJUGATED TO A HAPTEN" granted on March 1, 1994, by assignment from the inventor David Berd recorded July 6, 1990 with the U.S. Patent and Trademark Office at Reel 5336, Frame 880, and on whose behalf and with whose assent the accompanying application for reissue is made, hereby offers to surrender said Letters Patent.

Thomas Jefferson University

Date: March 5, 1998

By: _____

Name, Title JUSSI J. SAUKKONEN, MD
Dean, College of Graduate Studies and
Vice President for Science Policy, Technology
Development and International Affairs

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D B Peck D B Peck

Name (Print) Signature

MAR 6 1998

PATENT & TRADEMARK OFFICE

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Assignee: Thomas Jefferson University

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

ASSENT TO REISSUE

Thomas Jefferson University, a non-profit organization existing under the laws of Pennsylvania, and having a place of business at 11th and Walnut Streets, Philadelphia, Pennsylvania. and assignee of the entire right, title, and interest in U.S. Patent 5,290,551, for "TREATMENT OF MELANOMA WITH A VACCINE COMPRISING IRRADIATED AUTOLOGOUS MELANOMA TUMOR CELLS CONJUGATED TO A HAPTEN" granted on March 1, 1994, hereby assents to the accompanying application to reissue said patent.

Thomas Jefferson University

Date: March 5, 1998

By: _____
Name, Title

JUSSI J. SAUKKONEN, MD
Dean, College of Graduate Studies and
Vice President for Science Policy, Technology
Development and International Affairs

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D B Peck

Name (Print)

Signature

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In re application of: David BERD

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(For Reissue of U.S. Patent No. 5,290,551)

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Assignee: Thomas Jefferson University

Honorable Commissioner of
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Washington, DC 20231

DECLARATION UNDER 37 C.F.R. §1.175

David BERD, the named inventor of United States Patent No. 5,290,551 ("the '551 Patent") and the applicant for reissue thereof declares that:

1. I am a citizen of United States residing at 125 Heacock Lane, Wyncote, Pennsylvania 19095.

2. I assigned the entire title to the '551 Patent, granted to me on March 1, 1994, for TREATMENT OF MELANOMA WITH A VACCINE COMPRISING IRRADIATED AUTOLOGOUS MELANOMA TUMOR CELLS CONJUGATED TO A HAPTEN to Thomas Jefferson University, by an assignment recorded in the United States Patent and Trademark Office on July 6, 1990, at Reel 5336, Frame 880.

3. I make this declaration under 37 C.F.R. §1.175 in support of the application for reissue.

4. I have reviewed and understand the contents of the above-identified specification, including the claims as amended.

5. I believe that I am the original, first and sole inventor of the subject matter which is described and claimed in the aforesaid '551 Patent and in the foregoing specification, including the claims as amended, and for which I solicit a reissue patent.

6. I acknowledge the duty to disclose information of which I am aware which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

7. Although I consider my invention a significant advance in the art, I believe the '551 Patent may be partially invalid because I may have claimed more than I had a right to claim in view of my abstract entitled "Tumor Inflammatory Response Induced by Immunization with Autologous Melanoma Cells Conjugated to Dinitrophenol (DNP)," *Proc. Am. Assoc. Cancer Res.*, v. 30, March 1989 (Exhibit A). This publication was not cited to

the Patent Office, although it was published more than one year prior to May 8, 1990, the filing date of U.S. application Ser. No. 520,649 ("the '649 application"), which is the parent of the application Ser. No. 985,334 ("the '334 application"). The '334 application issued as the '551 Patent. The Abstract was also presented at the 80th Annual Meeting of the American Association for Cancer Research ("the AACR Meeting") held May 24-27, 1989 in San Francisco, which was less than one year before the May 8, 1990 filing date.

8. At the time the '649 application was being prepared for filing in the period immediately prior to May 8, 1990, I believed that the Abstract was made public at the AACR Meeting in San Francisco, on or about May 24, 1989. Consequently, I believed that the meeting date was the critical date for filing my application. I did not appreciate that the AACR Meeting abstract was published before the actual meeting date, nor that the actual date of publication of my abstract was prior to May 8, 1989. Thus, I did not advise my attorneys, the Patent Office or anyone else as to any publication date for the Abstract that was earlier than the AACR Meeting, May 24-27, 1989. I now realize that it was an error to overlook the Abstract publication date, and it was an error not to inform the Patent Office concerning this prior art. This error was made without any deceptive intent.

9. The error came to my attention when the Abstract was cited by the Examiner in a related application, Ser. No. 08/203,004 filed February 28, 1994.

I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code, and may jeopardize the validity of the application or any patent issuing therefrom.

FULL NAME AND RESIDENCE OF INVENTOR

LAST NAME: **Berd** FIRST NAME: **David** MIDDLE NAME:

CITY: **Wyncote** STATE OR FOREIGN COUNTRY: **Pennsylvania** COUNTRY OF CITIZENSHIP: **U.S.**

POST OFFICE ADDRESS:**125 Heacock Lane** CITY:**Wyncote** STATE OR COUNTRY:**PA** ZIP CODE: 19095

Date: 3/5/98

David Berd

David BERD, Inventor

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B Peck — Name (Print)

Signature

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PRELIMINARY AMENDMENT

Sir:

This preliminary amendment is part of the accompanying reissue application. Kindly make the amendments shown herein, prior to the examination of the application. As explained in the accompanying declaration by inventor David Berd, reissue of the patent with these amendments is sought because the patentee may have claimed more than he had a right to claim. The amendments do not add new matter. Consideration and allowance is respectfully requested.

IN THE CLAIMS:

(All claims are reproduced for the Examiner's convenience. Claims that have not been amended are indicated as "Unchanged.")

1. (Unchanged) A vaccine useful for the treatment of melanoma comprising irradiated autologous melanoma cells conjugated to a hapten, said hapten selected from the group consisting of dinitrophenyl, trinytrophenyl, and N-iodoacetyl-N'-5 sulfonic 1-naphtyl ethylene diamine; and mixed with an immunological adjuvant, wherein said immunological adjuvant is *Bacille Calmette-Guerin*.

2. (Unchanged) A method for treating melanoma comprising administering cyclophosphamide followed by intradermal administration of a therapeutically effective amount of the vaccine of claim 1.

Please add the following new claims:

--3. (New) The vaccine of claim 1, wherein said autologous melanoma cells are cryopreserved.

--4. (New) The vaccine of claim 1, wherein said autologous melanoma cells are irradiated with a low radiation dose.

--5. (New) The method of claim 2, wherein said autologous melanoma cells are cryopreserved.

--6. (New) The method of claim 2, wherein said vaccine is injected at multiple sites per administration.

--7. (New) The method of claim 6, wherein said vaccine is injected into three contiguous sites on an upper arm or leg.

--8. (New) The method of claim 2, wherein said vaccine is administered to post-surgical melanoma patients.

--9. (New) The method of claim 2, wherein said vaccine is administered to stage four melanoma patients.

--10. (New) The method of claim 2, wherein said autologous melanoma cells are irradiated with a low radiation dose.

--11. (New) The method of claim 2, further comprising multiple intradermal administrations every 4 weeks.

--12. (New) The method of claim 2, further comprising more than two intradermal administrations.

--13. (New) A method for treating melanoma comprising administering cyclophosphamide followed by intradermal administration of a therapeutically effective amount of a vaccine composition comprising cryopreserved and irradiated autologous melanoma cells conjugated to a hapten, said hapten selected from the group consisting of dinitrophenyl, trinytrophenyl, and N-iodoacetyl-N'-5 sulfonic 1-naphtyl ethylene diamine and mixed with *Bacille Calmette-Guerin*.

--14. (New) The method of claim 13, wherein said vaccine is injected at multiple sites per administration.

--15. (New) The method of claim 14, wherein said vaccine is injected into 3 contiguous sites on an upper arm or leg.

--16. (New) The method of claim 13, wherein said vaccine is administered to post-surgical melanoma patients.

--17. (New) The method of claim 13, wherein said vaccine is administered to stage four melanoma patients.

--18. (New) The method of claim 13, wherein said autologous melanoma cells are irradiated with a low radiation dose.

--19. (New) The method of claim 13, further comprising multiple intradermal administrations every 4 weeks.

--20. (New) The method of claim 13, further comprising more than two intradermal administrations.--

REMARKS

Early and favorable consideration of this reissue application is respectfully requested.

Upon entry of this amendment, claims 1-20 will be pending in the application. New claims 3-20 have been added. New claim 3, 5, and 13 are supported at col. 4, lines 62-63, of U.S. Patent No. 5,290,551 (the '551 Patent) which teach "autologous, cryopreserved, irradiated ... tumor cells." New claims 4, 10 and 18 find support at col 3, line 50, of the '551 Patent, which teaches that autologous melanoma cells are "irradiated at 2500 R," a dose recognized in the art as a low radiation dose. Claims 6, 7, 14 and 15 find support at col 3, lines 40-41, of the '551 Patent, which teach that the vaccine composition can be injected "into 3 contiguous cites on the upper arms or legs." Claims 8 and 16 are supported at col. 5, lines 43-44, which teach that "tumors [are] excised from [the] patients before immunotherapy." Claims 9 and 17 are supported at col. 5, lines 4-6, teaching that tumor regression occurred "in lung and liver metastases" which are known in the art to occur only in stage four melanoma patients. Claims 11, 12, 19 and 20 are supported at col 3, lines 32-33, and col 6, lines 14-17, which teach that the vaccine is "reinjected every 4 weeks" and Example 4 (col 6, lines 34-64) which teaches that the vaccine is administered three times.

As explained in the accompanying reissue declaration, the inventor and the patentee believes that the '551 patent may claim more than it had the right to claim. This reissue application and preliminary amendment is presented to address the prior art not cited during prosecution. More particularly, an Abstract authored by inventor David Berd (Tumor Inflammatory Response Induced by Immunization with Autologous Melanoma Cells Conjugated to Dinitrophenol (DNP); *Proc. Am. Assoc. Cancer Res.*, v. 30, March 1989) was published more than one year before the earliest filing date of the '551 patent. As explained in the accompanying declaration, this Abstract was not cited because the early publication date was not appreciated, nor was there any deceptive intent in omitting to cite the Abstract. The circumstances surrounding the error and lack of deceptive intent are set forth in the accompanying declaration.

CONCLUSION

It is believed that the claims warrant allowance and such action is earnestly solicited. If the Examiner disagrees, or believes for any reason that the direct contact with applicant's attorney would advance prosecution of this application, he or she is invited to telephone at the number given bellow.

Respectfully submitted,

Nada Jain, Ph.D.
Registration No. 41,431
Attorney for Applicants

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New York, N.Y. 10022
(212) 527-7700

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